UNIVERSITI PUTRA MALAYSIA

NUTRIENT COMPOSITION AND HYPOCHOLESTEROLEMIC EFFECT OFBLACK SEEDS (NIGELLA SATIVA L.) AND ITS OIL

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FPSK(M) 2005 16

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By

GHANYA NAJI AL-NAQEEB

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

May 2005

To may parent
To Al-Naoeep family
To all people from Saber

To my country, Republic of Yemen
To those who are really very hard working in any where

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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GHANYA NAJI AL-NAQEEB

May 2005

Chairman: Associate Professor Maznah Ismail, Ph.D.

Faculty : Medicine and Health Sciences

Nigella sativa Linn. (Ranuculacea) popularly known as the black seed or Habbatus Sauda is a herb that has traditionally been used for centuries in many parts of the world. It has gained popularity due to its potential health benefits. This study was carried out to investigate the nutrient composition and *in vivo* hypocholesterolemic effect of the black seeds cultivated in Yemen and its oil. The nutrient composition of three different samples of *Nigella sativa* seeds from three different regions in Yemen, namely Marib, Sadah and Taiz were studied. Proximate analysis, total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) were determined by the Association of Official Analytical Chemists methods (AOAC). Mineral analysis was carried out using Atomic Absorption spectrophotometer (AAS). Fatty acid methyl esters composition was determined using Gas Chromatography (GC), vitamin E (α-tocopherol) using HPLC and antioxidant activity by ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods. Results show that the three samples of *Nigella sativa* seeds have high content of fat which were 38.4 ± 2.1, 37.7 ± 0.1, and 36.8 ± 1.4% in Sadah, Marib, and Taiz, respectively. In addition, *Nigella sativa* seeds have a high content of TDF (36.88 ± 1.44, 26.50 ± 1.05, 30.40 ± 1.06% for Marib, Sadah and Taiz

samples, respectively), IDF (27.10 ± 0.55, 20.56 ± 1.16, 22.40 ± 1.40 % for Marib, Sadah and Taiz samples, respectively) and SDF (8.90 ± 1.17, 6.50 ± 0.60, 8.13 ± 0.71 % for Marib, Sadah and Taiz samples, respectively).

Nigella sativa seeds are also rich in calcium, magnesium, potassium, phosphorus and iron. The major fatty acids in *Nigella sativa* oil extracts from n-hexane were oleic and linolenic which exist as unsaturated fatty acids. Palmitic, stearic and myristic acids were the main saturated fatty acids in all three samples. Oil extracted from the seeds showed higher antioxidant activity compared to α-tocopherol, and was rich in vitamin E (120 ± 0.15, 170 ± 0.40 and 290 ± 1.5 mg/100g for Marib, Sadah and Taiz samples, respectively).

The effect of *Nigella sativa* seed powder and its oil on hypercholesterolemia-induced rabbits was also studied for 8 weeks. A significant reduction ($p< 0.05$) of total cholesterol (TC) and low density lipoproteins (LDL) in *Nigella sativa* powder (NSP) and *Nigella sativa* oil (NSO) groups were observed at weeks 2, 4, 6 and 8. Treatment of rabbits with NSP and NSO showed a significant increase ($p>0.05$) in plasma HDL levels at weeks 4, 6 and 8 of treatment. A significant decreased ($p<0.05$) of malondialdehyde (MDA) concentration in erythrocyte and plasma was observed in NSP and NSO groups. There was a significant increase of total plasma antioxidant status (TAS) ($p>0.05$). NSP and NSO groups also showed significant reduction of serum alanine aminotransferase (ALT) and creatinine concentrations ($p<0.05$). Plaque formation was significantly inhibited in the aorta, and the tunica intima to tunica media ratio was significantly decreased ($p<0.05$).

In conclusion, this study indicates that the seed of *Nigella sativa* cultivated in Yemen and its oil contain several nutrients and antioxidants with potential health values such as hypocholesterolemic effect, antioxidant effect and anti atherogenic effect.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KOMPOSISI NUTRIEN DAN KESAN HIPOKOLESTEROLEMIK "BIJAN HITAM" (*NIGELLA SATIVA* L.) DAN MINYAKNYA

Oleh

GHANYA AL-NAQEEB

May 2005

Pengerusi: Profesor Madya Maznah Ismail, Ph.D.

Fakulti : Perubatan dan Sains Kesihatan

Nigella sativa L. (Ranuculacea) yang biasanya dikenali sebagai bijan hitam atau Habbatus Sauda merupakan sejenis herba yang digunakan secara meluas sejak berkurun lamanya di kebanyakan negara di dunia disebabkan oleh potensi kesihatannya. Walau bagaimanapun, lebih banyak maklumat saintifik diperlukan bagi menyokong potensi kesihatan ini. Kajian ini dijalankan untuk mengkaji komposisi nutrien serta kesan hipokolestrolemik bijan hitam yang ditanam di Yemen dan minyaknya secara *in vivo*. Komposisi nutrien telah dianalisis daripada tiga sampel biji *Nigella sativa* dari tiga kawasan iaitu Marib, Sadah dan Taiz. Analisis proksimat, jumlah fiber diet (TDF), fiber diet tidak larut (IDF) dan fiber diet larut telah ditentukan melalui kaedah AOAC. Analisis mineral (besi, kalsium, magnesium, kalium, natrium, fosforus dan zink) telah dijalankan dengan menggunakan sistem nyalaan, Spektrofotometer Serapan Atom (AAS). Kandungan asid lemak metil ester telah ditentukan melalui kaedah kromatografi gas (GC-MS), kandungan vitamin E (α-tokoferol) menggunakan kromatografi cecair prestasi tinggi (HPLC), dan kandungan antioksidan melalui kaedah ferik tiosianat (FTC) dan asid tiobarbiturik (TBA).

Keputusan menunjukkan ketiga-tiga sampel biji *Nigella sativa* mengandungi kandungan minyak yang tinggi, iaitu 37.7 ± 1.5, 38.4 ± 2.1 dan 36.8 ± 1.4%, masing-masing dalam sampel Marib. Sadah dan Taiz. *Nigella sativa* juga mempunyai kandungan TDF yang tinggi, iaitu 36.88 ± 1.44, 26.50 ± 1.05, 30.40 ± 1.06%, IDF, iaitu 27.10 ± 0.55, 20.56 ± 1.16, 22.40 ± 1.40 %, dan SDF, iaitu 8.90 ± 1.17, 6.50 ± 0.60, 8.13 ± 0.71% masing-masing untuk sampel Marib, Sadah dan Taiz.

Nigella sativa kaya dengan kalsium, magnesium, kalium, fosforus dan zat besi. Komposisi asid lemak metilester bagi minyak yang diekstrak menggunakan *n*-heksana ialah oleik dan linoleik yang hadir sebagai asid lemak tak tepu yang utama. Asid palmitik, stearik dan miristik merupakan asid lemak tepu yang utama dalam ketiga-tiga samepl dari Marib, Sadah dan Taiz. Minyak daripada biji *Nigella sativa* telah menunjukkan aktiviti antioksidan yang lebih tinggi berbanding α-tokoferol rujukan, dan didapati kaya dengan vitamin E (120 ± 0.15, 170 ± 0.40 dan 290 ± 1.5 mg/100g masing-masing bagi sampel Marib, Sadah dan Taiz).

Kesan serbuk biji dan minyak bijan hitam kepada arnab yang diaruh hiperkolestrolemik telah dikaji selama 8 minggu. Penurunan yang signifikan ($p<0.05$) bagi jumlah kolesterol (TC), lipoprotein ketumpatan rendah (LDL) dalam kumpulan diberi serbuk *Nigella sativa* (NSP) dan kumpulan yang diberikan minyak *Nigella sativa* (NSO) berlaku pada minggu rawatan ke 2, 4, 6 dan 8. Arnab yang dirawat dengan NSP dan NSO menunjukkan peningkatan yang signifikan ($p<0.05$) pada tahap lipoprotein berketumpatan tinggi (HDL) dalam plasma pada minggu ke 4, 6 dan 8. Tiada perubahan yang signifikan didapati bagi paras trigliserida (TG) sepanjang masa rawatan. Kedua-

dua kumpulan NSP dan NSO menunjukkan penurunan yang signifikan ($p<0.05$) bagi kepekatan malondialdehid (MDA) dalam eritrosit dan plasma. Jumlah status antioksidan plasma (TAS) juga telah meningkat secara signifikan ($p<0.05$). NSP dan NSO pula menunjukkan penurunan yang signifikan bagi kepekatan alanin aminotransferase (ALT) dan kreatinin dalam serum ($p<0.05$).

Pembentukan plak juga telah direncat dan nisbah tunika intima kepada tunika media pada lapisan dinding aorta telah menurun secara signifikan ($p<0.05$). Kesimpulannya, kajian ini telah menunjukkan bahawa serbuk biji *Nigella sativa* yang ditanam di Yemen dan minyaknya mengandungi pelbagai nutrien dan antioksidan yang dapat memberikan kesan hipokolestrolemik, antioksidan dan anti-aterogenik.

ACKNOWLEDGEMENTS

I owe first and foremost my profound gratitude to almighty ALLAH, the source of all inspiration and help, and without whose assistance, this study would not have come into existence.

Deep obligation and indebtedness and most sincere gratitude are offered to my supervisor Associate Professor Dr. MAZNAH ISMAIL for her continuous guidance during all stages of my research work and for her willingness to help, listen and assist in every way, in the midst of her heavy responsibilities. I also record my sincere appreciation to the committee members: Dr. NORHAIZAN MOHD. ESA and Dr. ZULKHAIRI HJ AMOM, for their guidance, valuable advice and precious evaluation and valuable comments.

I sincerely appreciate and acknowledge staff, laboratory assistants and graduate students at Department of Nutrition and Health Sciences for their help and cooperation. Finally I am deeply obligated and thankful to my father, my mother, my sisters, brothers, all my friends and Al – Barra for their great help, patience, support and their encouragement over the course of this thesis.

I certify that an Examination Committee met on 30th May 2005 to conduct the final examination of Ghanya Naji Al-Naqeep on her Master of Science thesis entitled "Nutrient Composition and Hypocholesterolemic Effect of *Nigella sativa* L." in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Sciences. The members of the Supervisory Committee are as followed:

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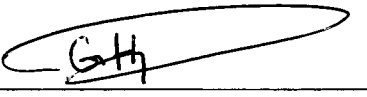
Date: 11 AUG 2005

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



GHANYA NAJI AL-NAQEEP

Date: 18/7/2005

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Apo	Apolipoprotein
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
ANOVA	One Way ANOVA
CHD	Coronary heart disease
CCL_4	Carbon tetrachloride
FTC	Ferric Thiocyanate
GC	Gas Chromatography
GGT	Gamma Glutamyltranspeptidase
HDL	High density lipoprotein
HMG-CoA	3-hydroxy-3-methylglutary l Coenzyme - A
IDF	Insoluble Dietary Fibre
IDL	Intermediate Density Lipoprotein
LCAT	Lecithin: Cholesterol Acyltransferase
LDL	Low Density Lipoprotein
LDH	Lactate Dehydrogenase
MDA	Malondialdehyde
min	Minute
NC	Negative control
PKC	Palm kernel cake
NSO	*Nigella Sativa* Seed Oil
SFA	Saturated Fatty Acid
SDF	Soluble Dietary Fibre
ST	Simvastatin

TAS	Total Antioxidant Status
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Acid Reactive Substances
TDF	Total Dietary Fiber
VLDL	Very Low Density Lipoprotein
USFA	Unsaturated Fatty Acid
Wk	Week

CHAPTER 1

INTRODUCTION

Cholesterol is an essential constituent of all animal cells, without it the living cells would not function properly and the organism would die (Saraswathi *et al.*, 1997). It has been documented that high plasma cholesterol level (hypercholesterolemia) is one of the most important coronary risk factors. Cardiac morbidity and mortality are directly related to serum cholesterol levels (Chen *et al.*, 1999). Many investigations indicate that elevated serum cholesterol levels may modify the biochemical properties of blood components and arterial intima, thus enhancing the development of atherosclerosis (Wallidus *et al.*, 1993).

It was reported that hypercholesterolemia induces a free radical mediated lipoprotein peroxidation and causes oxidative stress. This stress results from the imbalance between the production of free radicals and the effectiveness of the antioxidant defense system. The activity of free radicals is countered by a system of antioxidant defenses, of which vitamin E is the major chain breaking lipophilic antioxidant in tissues and plasma. In addition to vitamin E, tissues and erythrocyte enzymes contribute to the cell antioxidant defense mechanism. These include superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px), which detoxify H_2O_2 and convert lipid hydroperoxides to nontoxic alcohol. Disorders in these erythrocyte enzyme activities have been reported in subjects with cardiovascular disease (Halliwel *et al.*, 1996).

Reduction of cholesterol level was the main target in preventing the development of coronary heart disease (CHD). Health care plans have also included cholesterol reduction and dietary changes as a way to protect against CHD (Abajo *et al.*, 1993).

There is great interest in choosing the best diet to maintain desirable serum lipid levels, which may protect against heart disease. High levels of serum total cholesterol, low-density lipoprotein cholesterol (LDL), and triacylglycerol levels have been associated with increased risk of heart disease, while high levels of high-density lipoprotein cholesterol (HDL) have been associated with decreased risk of heart disease (Nicolosi *et al.*, 2001). Thus, a dietary pattern that most effectively lowers TC, LDL, and triacylglycerol levels, while maintaining or increasing HDL would be desirable. However, the ideal dietary intervention to improve serum lipids and reduce heart disease risk remains controversial (Nicolosi *et al.*, 2001).

It has been shown that increased consumption of omega-3 polyunsaturated fatty acids improves endothelium-dependent relaxation and protects against the development of atherosclerotic cardiovascular diseases (Prasad *et al.*, 1997; Nicolosi *et al.*, 2001). Studies have shown that vitamin E administered to hypercholesterolemic rabbits significantly reduced the plasma LDL and vessel wall oxidation, a potential beneficial action of vitamin E in hypercholesterolemic patients (Jorge *et al.*, 1998). Consumption of products containing dietary fiber has increased due to the health benefits that they offer (Khaw *et al.*, 1987). Insoluble as well as soluble dietary fibers have been associated with cholesterol lowering properties, which can be effective in lowering the risk of cardiovascular disease. Recent findings have demonstrated that the insoluble fiber derived from some fruits and